UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL EFFECTS OF CALCIUM ON OIL PALM SEEDLING GROWTH AND SUPRESSION OF* Ganoderma *DISEASE

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By

NURUL MAYZAITUL AZWA JAMALUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2021

DEDICATION

This thesis is dedicated

to

My understanding and lovely husband: Mohd Ezmir Shafiq bin Zainuddin

My beloved son: Ezekiel Mikhael

My beloved daughter: Eva Mikhayla

My understanding and supportive parents: Jamaludin bin Ahmad and Sahemah binti Amir

for their love and patience.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Mohamed Hanafi bin Musa, PhD
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Basal stem rot (BSR) disease caused by the *Ganoderma* species was a severe problem to the oil palm (*Elaeis guineensis* Jacq.) industry. Nutrients were commonly used in the fertilizers to enhance plant growth and/also to protect against abiotic and biotic stresses. Therefore, manipulation of plant nutrients, especially calcium (Ca) as an option for-prevention of BSR disease in oil palm. Thus, the goals of this research were to determine the optimum concentration of Ca in oil palm seedlings, to determine the effects of different Ca sources on vegetative growth in oil palm seedlings, to assess the effects of Ca formulation treatment in the nursery to suppress *Ganoderma* infection in oil palm seedlings and to investigate the Ca formulation in controlling *Ganoderma* disease in oil palm under field condition. The optimum concentration of Ca was determined by growing of oil palm seedlings using a modified Hoagland's nutrient solution containing different concentrations of Ca for three months in a controlled environment. Concentration of 1,000 ppm of Ca from $CaCl_2$ in the nutrient solution provided the best growth performance and development of oil palm seedlings. Subsequently, a nursery evaluation was conducted to determine the best sources of Ca and accompanying anions with oil palm seedlings planted on a Beach Ridges Interspersed with Swales (BRIS) soil for six months in MPOB nursery, Bandar Baru Bangi. An increased in seedling height, girth, chlorophyll content, and total biomass was observed on a treatment of 1,000 ppm Ca as $CaSO_4$. Further, oil palm seedlings were pre-treated with formulated fertilizer containing 1,000 ppm Ca as $CaSO_4$, then challenged with *G. boninense* PER 17 using rubber wood blocks (RWBs) sitting technique during the nursery trial (12 months) and baiting technique in the field trial (21 months). In nursery and field trials, at this concentration, the formulated fertilizer provided a better growth performance and at the same time to prevent BSR, by considerably reducing 52.8 and 81.1%, disease incidence (DI), respectively. Hence, Ca supplementation in the fertilizer could provide an alternate prevention program for BSR disease in oil palm plantation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PEMAKANAN KALSIUM TERHADAP PERTUMBUHAN PADA ANAK KELAPA SAWIT DAN PENGURANGAN PENYAKIT *Ganoderma*

Oleh

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Penyakit reput batang pangkal (BSR) yang disebabkan oleh spesies *Ganoderma* merupakan masalah yang teruk kepada industri kelapa sawit (*Elaeis guineensis* Jacq.). Nutrien biasanya digunakan dalam baja untuk meningkatkan pertumbuhan tanaman dan juga untuk melindungi daripada tekanan abiotik dan biotik. Oleh itu, manipulasi nutrien tumbuhan khususnya kalsium (Ca) sebagai pilihan untuk mencegah penyakit BSR pada kelapa sawit. Oleh yang demikian, matlamat penyelidikan ini adalah untuk menentukan kepekatan optimum Ca dalam anak pokok kelapa sawit, untuk menentukan kesan sumber Ca yang berbeza terhadap pertumbuhan vegetatif dalam anak pokok kelapa sawit, untuk menilai kesan rawatan formulasi Ca di nurseri untuk menyekat jangkitan *Ganoderma* pada anak pokok kelapa sawit dan untuk menyiasat formulasi Ca dalam mengawal penyakit *Ganoderma* pada kelapa sawit di ladang. Kepekatan Ca yang optimum ditentukan dengan menanam anak pokok kelapa sawit menggunakan larutan nutrien Hoagland yang diubah suai dan mengandungi kepekatan Ca yang berbeza selama tiga bulan dalam persekitaran yang terkawal. Kepekatan 1,000 ppm Ca daripada $CaCl_2$ dalam larutan nutrien memberikan prestasi pertumbuhan dan perkembangan terbaik pada anak pokok kelapa sawit. Kemudian, penilaian di nurseri telah dijalankan untuk menentukan sumber Ca yang terbaik dan iringan bersama anion pada anak pokok kelapa sawit yang telah ditanam di atas tanah *Beach Ridges Interspersed with Swales* (BRIS) selama enam bulan di nurseri MPOB, Bandar Baru Bangi. Peningkatan ketinggian anak pokok kelapa sawit, lilitan, kandungan klorofil, dan jumlah biojisim telah diperhatikan pada rawatan 1,000 ppm Ca sebagai $CaSO_4$. Kemudian, anak pokok kelapa sawit telah dirawat terlebih dahulu dengan baja formula yang mengandungi 1,000 ppm Ca sebagai $CaSO_4$, seterusnya telah dicabar dengan *G. boninense* PER 17 menggunakan teknik duduk blok kayu getah (RWB) semasa percubaan di nurseri (12 bulan) dan teknik mengumpan dalam percubaan di ladang (21 bulan). Hasil ujian di nurseri dan ladang pada kepekatan ini, baja yang dirumus memberikan prestasi pertumbuhan yang lebih

baik dan pada masa yang sama dapat mencegah penyakit BSR, dengan ketara mengurangkan sebanyak 52.8 dan 81.1%, kejadian penyakit (DI), masing-masing. Oleh itu, suplemen Ca dalam baja boleh menyediakan program pencegahan alternatif untuk penyakit BSR di ladang kelapa sawit.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requierement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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LIST OF ABBREVIATIONS

%	Percentage
µ	Micro
µg	Microgram
µm	Micrometer
µmol	Micromole
^{0}C	Degree celcius
AA	Auto analyzer
AAS	Atomic absorption spectroscopy
ANOVA	One-way variance analysis
AUDPC	Area under the progressive disease curve
B	Boron
BF	Basic fertilizer
BRIS	Beach Ridges Interspersed with Swales
BSR	Basal Stem Rot
Ca	Calcium
$Ca(NO_3)_2$	Calcium nitrate
$Ca(OH)_2$	Calcium hydroxide
CaC_2O_4	Calcium oxalate
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
cm	Centimetre
CRD	Completely randomized design
Cu	Copper

DF	Dilution factor
DI	Disease incidence
DIR	Director-like gene
DR	Disease reduction
DSIB	Disease severity index of bole
DSIF	Disease severity index of foliar
DSIR	Disease severity index of root
E. guinensis Jacq.	*Elaeis guineensis* Jacqueline
E. odora	*Elaeis odora*
E. olifera Cortez	*Elaeis olifera* Cortez
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Federal Land Development Authority
FRIM	Forest Research Institute Malaysia
g	Gram
G. boninense	*Ganoderma boninense*
GSM	*Ganoderma* selective medium
G-unit	Guaiacyl
H_2SO_4	Sulfuric acids
HCl	Hydrochloric acid
H-Unit	P-coumaryl alcohol
$Jmol^{-1}$	Joule per mole
K	Potassium
kg	Kilogram
KH_2PO_4	Monopotassium phosphate

KNO_3	Potassium nitrate
L	Liter
LSD	Least significant difference
M	Mole
MAI	Months after inoculation
MEA	Malt extract agar
Mg	Magnesium
mg	Milligram
mg	Milligram
$MgSO_4$	Magnesium sulphate
mM	Millimole
mm	Millimeter
Mn	Manganese
Mo	Molybdate
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Certification Council
N	Nitrogen
N	Normality
NaOH	Sodium hydroxide
nm	Nanometer
NUF-WLP	Water Leach Purification and Neutralization Underflow
P	Phosphorus
PDA	Potato Dextose Agar
POD	peroxidase
PPB	Plant Pathology Laboratory, Plant Pathology and Biosafety Unit

ppm	Part per million
R&D	research and development
RCBD	Randomized complete block design
RWB	Rubber wood block
SA	Salicylic acid
SAS	Statistical analysis software
SEA	Southeast Asia
SFS	Severity of foliar symptom
Si	Silicon
sp.	Species
SPAD	Chlorophyll meter
spp.	Species
S-unit	Sinapyl alcohol
TEM	Transmission electron microscopy
USR	Upper Stem Rot
v	Volume
w	Weight
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background Information

Calcium (Ca) is important macronutrients required for plant growth and production in both stress and non-stress conditions. It has a particular role among the mineral nutrient components which has been included in the category of ten macronutrients for a century. Calcium plays a key role in the process of maintaining the structural and plant membrane's functional integrity, stabilizing cell wall structures, regulating ion transport and selectivity, and controlling ion-exchange behavior as well as cell wall enzyme activity (Marschner, 1995; Rengel, 1992). However, the intake of Ca in plants is often so minimal that it is considered a micronutrient. The availability of Ca depends on its concentration in the soil solution as well as on chemical properties, including acidity and aluminum levels (Fageria *et al*., 2008; Ouertatani *et al*., 2011). Since calcium tends to be easily extracted from its membrane binding sites by other cations, these functions may be significantly impaired by decreased availability of calcium. This displacement was essentially counteracted by an increase in the external calcium concentration (Lynch and Läuchli, 1988).

The lack of mobility is one of Ca's peculiarities. It is transmitted through the xylem into the different plant organs after its absorption by the roots, but it is not dispersed *via* the phloem. Therefore, calcium cannot be remobilized from the older tissue to active growth zones as root absorption is disrupted. Calcium deficiency causes stunted growth of plants, curling of young leaves or leaves with necrotic leaf margins, and death of terminal buds and root tips. Generally, the plant's new growth and rapidly developing tissues are first affected. Maintaining an appropriate supply of calcium in soil solutions is therefore an essential factor in managing plant Ca deficiency.

1.2 Problem Statement

As with every other crop, the oil palm is often exposed to multiple pests and diseases. Basal Stem Rot (BSR) causes by fungus, *Ganoderma boninense* (*G. boninense*), is a major threat to oil palm industries in South East Asian countries, in particular in Malaysia and Indonesia, is one of the most extreme diseases (Naher *et al.*, 2013). Physical, biological and chemical controls have not been very successful because already visibly infected and subclinical palms may already have the disease established by the treatment is applied. The BSR disease is the leading cause of economic loss in oil palm plantations (Corley and Tinker, 2003). The disease is dangerous and has spread exponentially over the past few decades. Approximately 7.4% of BSR disease in Malaysia is reported, with a total area affected of 221, 000 ha (Idris *et al*., 2019). The BSR problem creates damages not only by a direct decrease in the number of oil palms, but also in the weight of the fruit bunch (Turner, 1981).

Several factors, such as palm age, previous crops, soil composition, nutrient quality and replanting techniques, have been reported to affect the progress of BSR disease in the field (Ariffin *et al.*, 2000). Detecting *G. boninense* infection requires early care of oil palms and avoids additional damage to the oil palm. The incidences of BSR from one palm to the next generation were confirmed. An integrated disease management approach to manage BSR is therefore important. Certain BSR disease control methods were developed and introduced in various oil palm plantations and smallholders in Malaysia in established standards and management strategies for replanting. Several cultural methods, mechanical, biological and chemical controls were proposed to decrease the occurrence of BSR disease in replanting, prolong the productivity of the infected palm and to slow the progression of the *G. boninense* outbreak. The biological solution cannot, however, avoid the production of fungal attacks on the oil palm. Moreover, it would seem necessary to pursue an alternate management solution which is more tolerant of the environment, considering the limitations of chemical pesticides. Through to the degree that all the critical plant nutrients impair plant health and their vulnerability to diseases, regulation of nutrient intake is an effective alternative technique (Agrios, 2005). Fertilizer nutrient manipulation has long been accepted as correlated with improvements in disease levels and plant yields.

This analysis focuses on fertilizer nutrients that use Ca as an alternative way to regulate the BSR. The most significant feature of this study is the role played by Ca in the resistance of oil palm to BSR disease from *G. boninense* fungal spores. The ideal solution for slowing the production of BSR in the oil palm is by the handling of the cell wall, particularly in lignin through the use of plant nutrients because of its major role in plant defense (Paterson *et al.*, 2009). Successful effects on powdery cucumber mildew (Adatia and Besford, 1986; Miyake and Takahashi, 1983) and *Pythium* root rots on cucumber have been shown to regulate this treatment on plant (Cherif *et al.*, 1992). The symptoms of BSR on clonal oil palm materials were greatly suppressed by the stabilization and enhancement of the cell walls of oil palm in the nursery (Sariah *et al.*, 1997; Sariah and Zakaria, 2000). Calcium oxalate (CaC_2O_4) induction in Lemma plants indicates that the crystal should be used to stock Ca for future requirements (Helper and Wayne, 1985). The Ca is required to release peroxidases linked to cell elongation regulation since they can rigidate the walls by crosslinking and their capacity to engage in lignin formation (Sticher *et al.*, 1981).

However, there is still little knowledge about the relation between Ca and the oil palm BSR disease. Responses to these questions are urgently needed considering the rising need for future studies. The present research was therefore intended to examine how the Ca affects nutritionally the reduction of BSR in the oil palm. In addition, Ca supplementation is often researched in order to determine the various possible forms and ability to resolve the issue of oil palm disease, if it is shown to be a feasible alternative therapy for BSR. In order to improve the high quality of palm oil and achieve high yield in future benign agriculture, the knowledge from this research can therefore be used.

1.3 Research objectives

Therefore, the objectives of this study were (i) To determine the optimum concentration of Ca in oil palm seedlings, (ii) To determine the effects of different Ca sources on vegetative growth in oil palm seedlings, (iii) To assess the effects of Ca formulation treatment in nursery to suppress *Ganoderma* infection in oil palm seedlings and (iv) To investigate the Ca formulation in controlling *Ganoderma* disease in oil palm.

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